

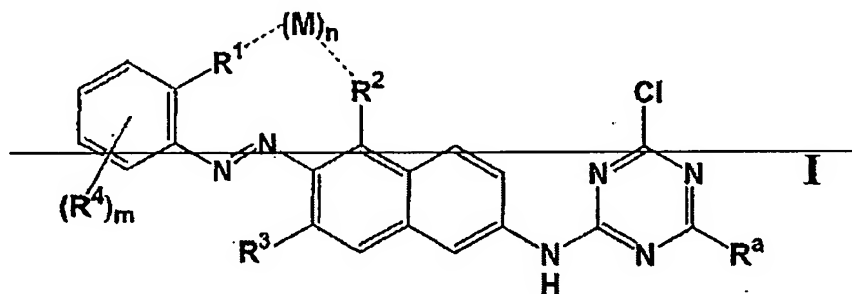
AMENDMENTS TO THE CLAIMS

This listing of the claims will replace all prior versions, and listings, of claims in the

Listing of Claims:

1. (Currently Amended) A method of visualizing a protein bound to a protein-binding membrane, said method comprising:

(1) staining at least one protein bound to a protein-binding membrane with an effective amount of a staining reagent comprising reactive brown 10, or a salt thereof ~~at least one compound of formula I:~~



wherein,

- ~~— R^a is selected from halogen and NH Ar;~~
- ~~— R¹ and R³ are independently selected from the group consisting of OH, COOH and SO₃H;~~
- ~~— R² is selected from the group consisting of OH and SH;~~
- ~~— R⁴ is selected from the group consisting of COOH, SO₃H, NH₂, NH(C₁-C₆)alkyl, NHacyl, NHAr, OH and O acyl;~~
- ~~— m is 0 or 1;~~
- ~~— M is a transition metal selected from the group consisting of chromium, manganese, iron, cobalt, nickel, copper, zinc, cadmium and combinations thereof;~~

~~_____ n is 0 or 1;~~

~~_____ indicates coordination to the transition metal M_i; and~~

~~_____ Ar is unsubstituted phenyl or substituted phenyl;~~

~~_____ wherein the substituents for Ar are selected from the group consisting halogen, NO₂, and
-SO₃H;~~

~~_____ or a salt of such a compound;~~

(2) incubating said protein bound to the protein-binding membrane with said staining reagent for a time interval sufficient to allow reaction of the protein with the staining reagent to yield a stained protein;

(3) destaining said staining reagent from the protein-binding membrane; and

(4) observing the stained protein;

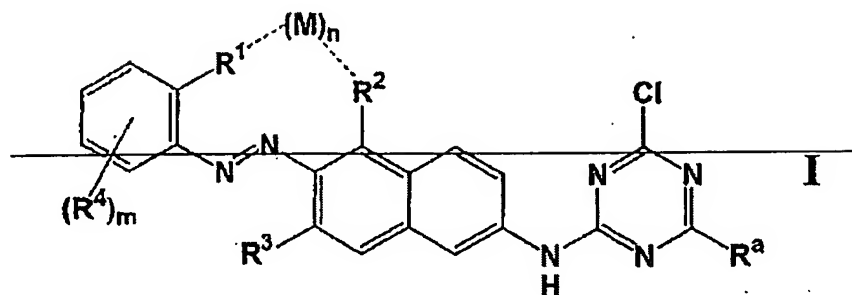
~~wherein about 2 ng of the stained protein are visually observable.~~

Claims 2-3. (Canceled)

4. (Original) The method of claim 1, wherein the protein-binding membrane is selected from the group consisting of nitrocellulose, nylon and polyvinylidene difluoride.

5. (Original) The method of claim 1 wherein the process is performed at room temperature.

6. (Currently Amended) A composition comprising at least one protein bound to a protein-binding membrane, which protein has been stained with a staining reagent comprising reactive brown 10, or a salt thereof ~~at least one compound of formula I:~~



wherein,

~~— R^a is selected from halogen and NH-Ar ;~~

~~— R^1 and R^3 are independently selected from the group consisting of OH , COOH and SO_3H ;~~

~~— R^2 is selected from the group consisting of OH and SH ;~~

~~— R^4 is selected from the group consisting of COOH , SO_3H , NH_2 , $\text{NH}(\text{C}_4\text{-C}_6)\text{alkyl}$, NHacyl , NHAr , OH and O-acyl ;~~

~~— m is 0 or 1;~~

~~— M is a transition metal selected from the group consisting of chromium, manganese, iron, cobalt, nickel, copper, zinc, cadmium and combinations thereof;~~

~~— n is 0 or 1;~~

~~— indicates coordination to the transition metal M ; and~~

~~— Ar is unsubstituted phenyl or substituted phenyl;~~

~~— wherein the substituents for Ar are selected from the group consisting halogen, NO_2 , and SO_3H ;~~

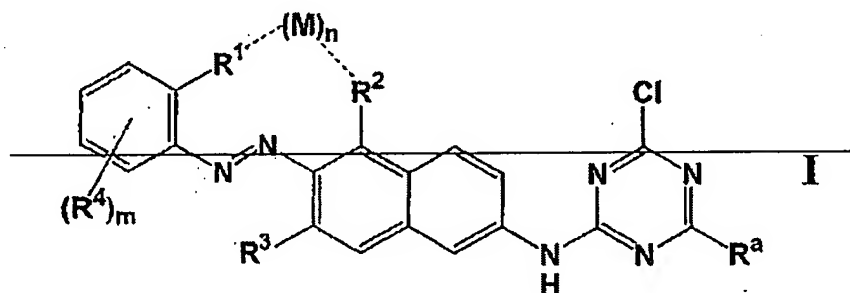
~~— or a salt of such a compound;~~

wherein said protein-binding membrane is capable of being destained, and wherein about 2 ng of said protein are visually observable when the protein-binding membrane is destained.

Claim 7. (Canceled)

8. (Currently amended) A method of reversing the staining procedure of claim 1, comprising:

(1) providing a protein-binding membrane having at least one protein spot bound thereto stained with a staining reagent comprising reactive brown 10, or a salt thereof ~~at least one compound of formula I:~~



wherein,

~~R^a is selected from halogen and -NH-Ar;~~

~~R¹ and R³ are independently selected from the group consisting of -OH, -COOH and -SO₃H;~~

~~R² is selected from the group consisting of -OH and -SH;~~

~~R⁴ is selected from the group consisting of -COOH, -SO₃H, -NH₂, -NH(C₁-C₆)alkyl, -NHacyl, -NHAr, -OH and -O-acyl;~~

~~m is 0 or 1~~

~~M is a transition metal selected from the group consisting of chromium, manganese, iron, cobalt, nickel, copper, zinc, cadmium and combinations thereof;~~

~~_____ n is 0 or 1;~~

~~_____ indicates coordination to the transition metal M; and~~

~~_____ Ar is unsubstituted phenyl or substituted phenyl;~~

~~_____ wherein the substituents for Ar are selected from the group consisting halogen, NO₂, and SO₃H;~~

~~_____ or a salt of such a compound;~~

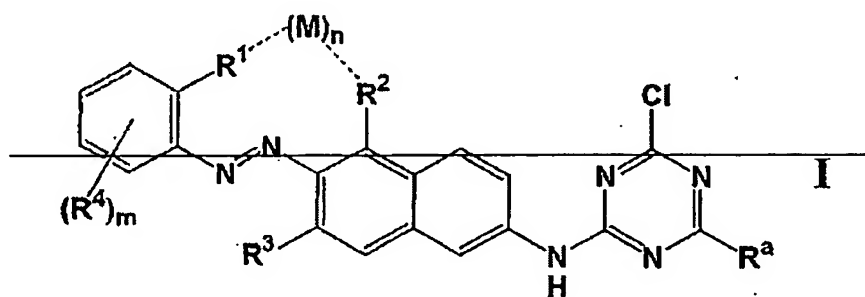
(2) incubating said protein-binding membrane with an aqueous alkaline solution; and

(3) washing the protein-binding membrane to remove the staining reagent.

Claim 9. (Canceled)

10. (Currently Amended) A method of quantifying a protein analyte, comprising:

(1) staining at least one protein analyte spot and a series of protein standard spots of known quantity bound to a protein-binding membrane with a staining reagent comprising reactive brown 10, or a salt thereof ~~at least one compound of formula I:~~



wherein;

~~_____ R^a is selected from halogen and NH Ar;~~

~~_____ R¹ and R³ are independently selected from the group consisting of OH, COOH and~~

~~SO₃H;~~

~~— R² is selected from the group consisting of OH and SH;~~

~~— R⁴ is selected from the group consisting of COOH, SO₃H, NH₂, NH(C₁-C₆)alkyl,
NHacyl, NHAr, OH and O-acyl;~~

~~— m is 0 or 1;~~

~~— M is a transition metal selected from the group consisting of chromium, manganese, iron,
cobalt, nickel, copper, zinc, cadmium and combinations thereof;~~

~~— n is 0 or 1;~~

~~— indicates coordination to the transition metal M; and~~

~~— Ar is unsubstituted phenyl or substituted phenyl;~~

~~— wherein the substituents for Ar are selected from the group consisting halogen, NO₂, and
SO₃H;~~

~~— or a salt of such a compound;~~

(2) incubating said protein analyte spot and said protein standard spots bound to the protein-binding membrane with the staining reagent for a time interval sufficient to allow reaction of the protein spot and said protein standard spots with the staining reagent;

(3) destaining the staining reagent from the protein-binding membrane, ~~wherein about 2 ng of said protein are visually observable;~~

(4) generating image quantification data for the known protein standard spots and for the protein analyte spot;

(5) constructing a standard calibration curve using the known concentrations of the protein standard and the corresponding image quantification data; and

(6) calculating a concentration for the protein analyte.

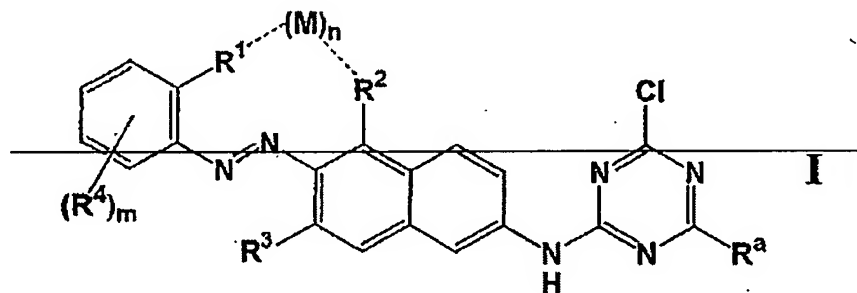
11. (Original) The method of claim 10, wherein the protein-binding membrane is selected from the group consisting of nitrocellulose, nylon and polyvinylidene difluoride.

12. (Original) The method of claim 10 wherein the protein standard is bovine serum albumin.

Claim 13. (Canceled)

14. (Currently Amended) A kit for visualizing a protein bound to a protein-binding membrane comprising, independently packaged within a single container:

- (1) one or more protein-binding membranes; and
- (2) a staining reagent comprising reactive brown 10, or a salt thereof at least one compound of formula I:



wherein,

— R^a is selected from halogen and NH-Ar ;

— R^1 and R^3 are independently selected from the group consisting of OH , COOH and SO_3H ;

— R^2 is selected from the group consisting of OH and SH ;

~~_____ R⁴ is selected from the group consisting of COOH, SO₃H, NH₂, NH(C₁-C₆)alkyl,
NHacyl, NHAr, OH and O acyl;~~

~~_____ m is 0 or 1;~~

~~_____ M is a transition metal selected from the group consisting of chromium, manganese, iron,
cobalt, nickel, copper, zinc, cadmium and combinations thereof;~~

~~_____ n is 0 or 1;~~

~~_____ indicates coordination to the transition metal M; and~~

~~_____ Ar is unsubstituted phenyl or substituted phenyl;~~

~~_____ wherein the substituents for Ar are selected from the group consisting halogen, NO₂, and
SO₃H;~~

~~_____ or a salt of such a compound,~~

~~wherein said one or more protein binding membranes are capable of being destained, and~~

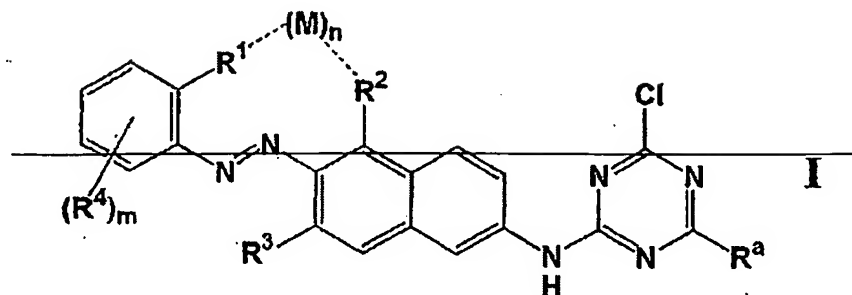
~~wherein said staining reagent is capable of making about 2 ng of a protein visually observable.~~

Claim 15. (Canceled)

16. (Currently Amended) A kit for quantifying an amount of a protein, comprising,
independently packaged within a single container:

(1) one or more protein-binding membranes;

(2) a staining reagent comprising reactive brown 10, or a salt thereof ~~a at least one
compound of formula I:~~



wherein,

~~— R^a is selected from halogen and —NH—Ar;~~

~~— R¹ and R³ are independently selected from the group consisting of —OH, —COOH and —SO₃H;~~

~~— R² is selected from the group consisting of —OH and —SH;~~

~~— R⁴ is selected from the group consisting of —COOH, —SO₃H, —NH₂, —NH(C₁–C₆)alkyl, —NHacyl, —NHAr, —OH and —O acyl;~~

~~— m is 0 or 1~~

~~— M is a transition metal selected from the group consisting of chromium, manganese, iron, cobalt, nickel, copper, zinc, cadmium and combinations thereof;~~

~~— n is 0 or 1;~~

~~— indicates coordination to the transition metal M; and~~

~~— Ar is unsubstituted phenyl or substituted phenyl;~~

~~— wherein the substituents for Ar are selected from the group consisting halogen, —NO₂, and —SO₃H;~~

~~— or a salt of such a compound; and~~

(3) a set of one or more solutions of a protein standard of known concentration[[,]]

wherein the staining reagent may be used to detect about 2 ng of a protein.

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Reply to Non-Final Office Action mailed September 30, 2008

Claim 17. (Canceled)